

In re Application of:
Short
Application No.: 09/848,095
Filed: May 3, 2001
Page 3

PATENT
Attorney Docket No.: DIVER1280-10

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for identifying bioactivities or biomolecules using high throughput screening of nucleic acid comprising:
 - a) generating an environmental gene library containing a plurality of clones in E.coli, wherein the nucleic acid for generating the library is naturally occurring and obtained from a mixed population of uncultured organisms;
 - b) transferring a plurality of the clones to [[a]] myceliate bacteria or myceliate fungi;
 - c) encapsulating a bioactive substrate and at least one clone transferred in b) in a gel microdroplet, wherein a bioactivity or biomolecule produced by the clone is detectable by a change in fluorescence of the substrate prior to contacting with the at least one clone as compared to after the contacting; and
 - d) screening the microdroplet with an assay or an analyzer that detects the presence therein of the change in fluorescence of the substrate, wherein the change indicates the identity of the bioactivity or biomolecule.
2. (Previously Amended) The method of claim 1, wherein the bioactivity is provided by an enzyme that is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

Claims 3-6 (Cancelled)

In re Application of:
Short

Application No.: 09/848,095

Filed: May 3, 2001

Page 4

PATENT

Attorney Docket No.: DIVER1280-10

7. (Original) The method of claim 1, wherein the gene library is an expression library.
8. (Previously Amended) The method of claim 7, wherein the expression library contains DNA obtained from extremophiles.
9. (Original) The method of claim 8, wherein the extremophiles are thermophiles.
10. (Previously Amended) The method of claim 9, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
11. (Original) The method of claim 1, wherein the bioactive substrate comprises C12FDG.
12. (Original) The method of claim 1, wherein the bioactive substrate comprises a lipophilic tail.
13. (Previously Amended) The method of claim 1, wherein the clones are heated before step c).
14. (Original) The method of claim 13, wherein the heating is at about 70°C.
15. (Previously Amended) The method of claim 14, wherein the heating occurs for about 30 minutes.
16. (Original) The method of claim 1, wherein the analyzer comprises a fluorescent analyzer.

In re Application of:
Short

Application No.: 09/848,095

Filed: May 3, 2001

Page 5

PATENT

Attorney Docket No.: DIVER1280-10

17. (Original) The method of claim 16, wherein the fluorescent analyzer is a FACS apparatus.
18. (Original) The method of claim 1, wherein the library is biopanned before step c).
19. Cancelled
20. (Previously Amended) The method of claim 1, wherein the myceliate bacteria is a *Streptomyces* sp.
21. (Original) The method of claim 20, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.
22. (Original) The method of claim 1, wherein the library is normalized before step b).
23. (Original) The method of claim 1, further comprising co-encapsulating an indicator cell in step c).
24. (Original) The method of claim 1, wherein the analyzer is a chromogenic analyzer.
25. (Original) The method of claim 1, wherein the assay is an immunoassay.
26. (Currently Amended) A method for identifying bioactivities or biomolecules using high throughput screening of nucleic acid comprising:

- a) generating an environmental gene library containing a plurality of clones in *E.coli*, wherein the nucleic acid for generating the library is naturally occurring and obtained from a mixed population of organisms;
- b) transferring a plurality of the clones to [[a]] myceliate bacteria or myceliate fungi;
- c) inserting a polynucleotide into the clones transferred in b), wherein the polynucleotide encodes a bioactive protein substrate, wherein a fluorescence change in the substrate is detectable in the presence of a bioactivity or biomolecule; and
- d) screening the clones with an assay or an analyzer that detects the presence therein of the fluorescence change in the substrate, wherein the fluorescence change in the substrate identifies the bioactivity or a biomolecule.

27. (Previously Amended) The method of claim 26, further comprising encapsulating the clone and the bioactive substrate prior to screening.

28. (Previously Amended) The method of claim 27, wherein the bioactivity is provided by an enzyme that is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, hailoperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

Claims 29-32 (Cancelled)

33. (Original) The method of claim 27, wherein the gene library is an expression library.

34. (Previously Amended) The method of claim 33, wherein the expression library contains DNA obtained from extremophiles.

35. (Original) The method of claim 34, wherein the extremophiles are thermophiles.
36. (Previously Amended) The method of claim 35, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
37. (Original) The method of claim 27, wherein the bioactive substrate comprises C12FDG.
38. (Original) The method of claim 27, wherein the bioactive substrate comprises a lipophilic tail.
39. (Previously Amended) The method of claim 27, wherein the clones are heated before step c).
40. (Original) The method of claim 39, wherein the heating is at about 70°C.
41. (Previously Amended) The method of claim 40, wherein the heating occurs for about 30 minutes.
42. (Original) The method of claim 27, wherein the analyzer comprises a fluorescent analyzer.
43. (Original) The method of claim 42, wherein the fluorescent analyzer is a FACS apparatus.

In re Application of:
Short

Application No.: 09/848,095

Filed: May 3, 2001

Page 8

PATENT

Attorney Docket No.: DIVER1280-10

44. (Original) The method of claim 27, wherein the library is biospanned before step b).

Claims 45-46 (Cancelled)

47. (Original) The method of claim 1, wherein the myceliate fungi is an *Actinomyces* sp.

48. (Original) The method of claim 1, wherein the myceliate bacteria is a *Streptomyces* sp.

49. (Original) The method of claim 47, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.

50. (Original) The method of claim 27, wherein the library is normalized before step c).

51. (Original) The method of claim 27, further comprising co-encapsulating an indicator cell in step c).

52. (Original) The method of claim 27, wherein the analyzer is a chromogenic analyzer.

53. (Original) The method of claim 27, wherein the assay is an immunoassay.

54. (Previously Amended) The method of claim 26, wherein the bioactive substrate is a fusion protein comprising a protein substrate flanked by two fluorescent proteins that upon contact cause a change in fluorescent signal from the clone, and wherein the effect of the presence of the biomolecule or bioactivity is to cause such contact.

In re Application of:

Short

Application No.: 09/848,095

Filed: May 3, 2001

Page 9

PATENT

Attorney Docket No.: DIVER1280-10

55. (Previously Amended) The method of claim 54, wherein the substrate is for a thioesterase.